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Application for Research Grant
(Use extra pages as needed)

Date: 1/27/75

1. Principal Investigator (give title and degrees):

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Professor of Medicine

2. Institution & address:

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University of Colorado Medical Center
4200 East 9th Avenue
Denver, Colorado 80220

3. Department(s) where research will be done or collaboration provided:

Department of Medicine
and
Webb-Waring Lung Institute

4. Short title of study:

Human Pulmonary Surfactant Function In Situ

5. Proposed starting date: 7/1/75

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

These will be achieved by applying three disciplines (pathology, physiology and physical chemistry) to surfactant in situ.

1) To determine the post-mortem distribution of the alveolar lining layer in healthy and diseased lungs of cigarette smokers using special fixation methods.

2) To determine the functions of surfactant in these lungs by postmortem physiological studies of airway closure, acinar clearance and alveolar collapse.

3) To determine the physical properties (especially wettability and adhesiveness) of pulmonary surfactant on dissected lung tissues in comparison to the properties on synthetic substrates.

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7a. Background information relevant to the objectives and working hypothesis:

The fact that surface phenomena contribute to lung retractive force, though reported by von Neergaard in 1929, was not generally appreciated till the direct demonstration of Pattle (1955) that the material lining alveoli had special properties. By squeezing bubbles from slices of lung at postmortem he obtained enough material to deduce one of its important physical properties--that its surface tension is "nearly zero" in vitro, that is to say, as the lining of a

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bubble outside the lung. His method of sampling the alveolar lining layer (as he called it) has been subsequently replaced in most laboratories by lung mincing or lung lavage methods, and the material so obtained has been subjected to extensive chemical analyses and surface balance studies. Valuable as these in vitro studies are, their indirect nature must be recognized and they must be correlated with the behavior of the lining layer in situ.

Because of the delicacy and inaccessibility of alveoli in the living lung very few direct physiological measurements have been made of alveolar wall deformation. In vivo observation of the lung surface (Wagner 1970) has not yielded quantitative information as to how this surface changes geometrically and our knowledge is largely limited to the results of measurements on quick frozen lung (Klingele and Staub 1970, Glazier 1967, Hughes et al 1970) or specially fixed tissue (Weibel and co-workers 1968, 1972, 1973). The latter have shown that alveoli are more polyhedral in shape than spherical, and that, at least in rat lungs fixed at three levels of inflation, the alveoli, rather than stretching with inflation, expand in a more complex manner perhaps best described as an "unpleating." The surface lining layer furthermore, rather than being a monolayer on a smooth surface, is distributed as sheets of variable thickness smoothing out tissue irregularities and as "pools of lining layer in crevasses." These authors have confirmed the finding of Klingele and Staub (1970) that below the physiologic range the alveoli "fold up from side to side as an accordion or concertina folds rather than by uniform decrease in all directions until they disappear as implicit in lung models based on the soap bubble analogy." Finally they have shown again in rat lungs, that the mean radius of curvature of expanded alveoli is about twice as large as in contracted alveoli and that the surface area-to-volume ratio was nearly constant instead of being inversely proportional to the radius of curvature as required by a spherical alveolar model.

How small airways 0.3 to 2.0 mm in diameter (alveolar ducts to 9th order bronchi) become narrowed and "close" is of vital importance to pulmonary physiology and chest medicine. Because of their location most investigations of small airway function have been indirect and their behavior has only recently begun to be understood (Macklem 1972). Direct intraluminal pressure measurements via tiny catheters have, thru the work of Macklem and his group (1965, 1967), revolutionized modern ideas of the mechanisms of airway obstruction and radically changed the meaning of standard ventilatory function tests (Mead 1970).

Excised lung tissue from experimental animals, meticulously dissected and studied by physiologic methods (Murtagh et al 1971, Menkes et al 1971, Hughes et al 1972), are being used to determine the relative contributions of intrinsic forces in airway walls, the luminal surface tension and the tissue forces of surrounding lung parenchyma. The recent review of Mead (1973) indicates the importance of these direct approaches to small airway function.

A recent direct attack on the problem of airway closure has been interpreted by the traditional concepts of how pulmonary surface forces operate, which we have examined above. Thus Macklem, Proctor and Hogg (1970) after recording

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P-V curves of air-filled dissected cat bronchioles before and after flushing with 2% Tween or dog lung extracts concluded "that surfactant lines and stabilizes bronchiles protecting against excessive radius changes with lung volume and air trapping. Their studies contradicted in some ways the finding of Cavagna et al (1967) that all airways are open at a transpulmonary pressure of zero, but the discrepancies were accounted for by "geometric factors" associated with distortion of airways consequent to the dissection procedure. The discrepancy between the results and those of others has been repeatedly referred to (Burger and Macklem 1968, Macklem et al 1970, Macklem 1971, Ingram et al 1974) and must be resolved.

Small airways, however, have many potential functions other than "staying open." A small airway's ability to convey liquid material originating from alveoli and, in particular to act as a conduit for the removal of toxic material from alveolar walls may be equally important. Unfortunately, although we know a fair amount about how ciliated airways are kept clear of unwanted material (Litt 1974), it is much less certain how alveolar tissue and small airway clearance mechanisms operate (Gross 1964, Staub 1966, Mendenhall 1972, Tucker et al 1973). New concepts are being developed to understand how cilia-free alveolar ducts and respiratory bronchiles clear themselves of noxious material. The importance of knowing whether or not surface active material is normally present and how it functions in the small airway lies in the possibility that changes in such material could well be produced by the inhalation of toxic substances. Thus DuBois and Rogers (1968) have concluded that the smaller bronchi are the most vulnerable sites for damage from inhaled particles. If surfactant is present in small bronchi, it can be shown (Sec. 9F) that toxic materials would be propelled toward the mouth by surface tension forces (i.e., without cough or ciliary action). If surfactant is absent or defective in the small airways of patients with emphysema, an obvious pathogenetic argument for the development of alveolar lesions early in this disease could be developed. Kilburn (1974) has presented evidence that certain small airways (those just at the junction of true alveolar tissue and the conduit bronchioles cleared by ciliary mechanisms), are a "no-man's land" as regards clearance, i.e. that they are in a region "cleared either way." It is clearly important to determine whether or not surface active material is present in such small airways and if it is capable of providing a clearance mechanism.

Finally, R.V. Ebert and M.J. Terracio have very recently (1975) shown that fresh surgical lung specimens from smokers show a loss of clara cells which may secrete a special sol (a strongly surface active colloid) in which cilia beat to move the overlying mucous gel. As Ebert says: "Unfortunately there is little direct evidence as to the nature of the surface coating of the bronchioles." Furthermore he emphasizes that when fixation via bronchi is used "the surface material is readily removed"--a point we consider in detail below (9.D.) The next page shows the delicacy of the surface we are considering as reproduced from page 9 of Ebert's paper.

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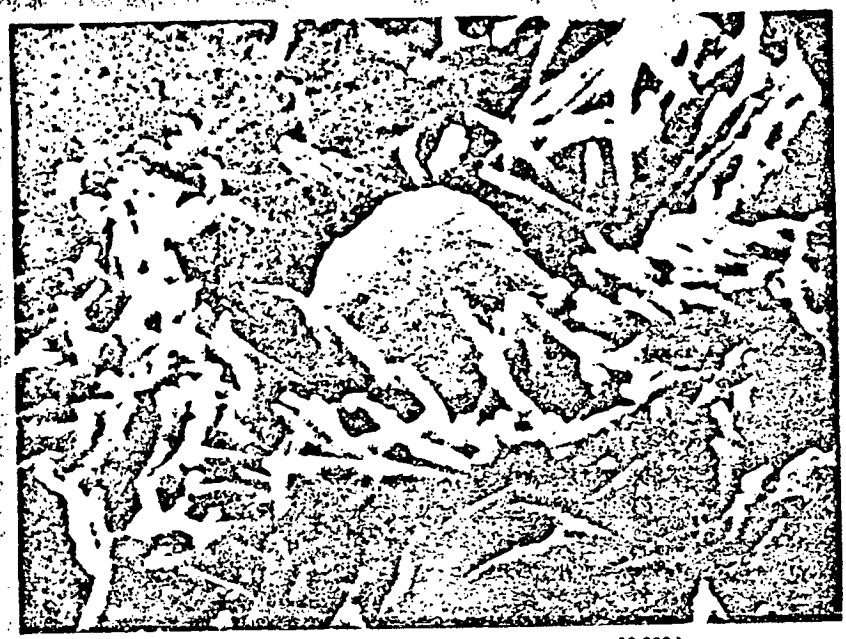

Fig. 5. A Clara cell surrounded by cilia. (Original magnification: × 10,000.)

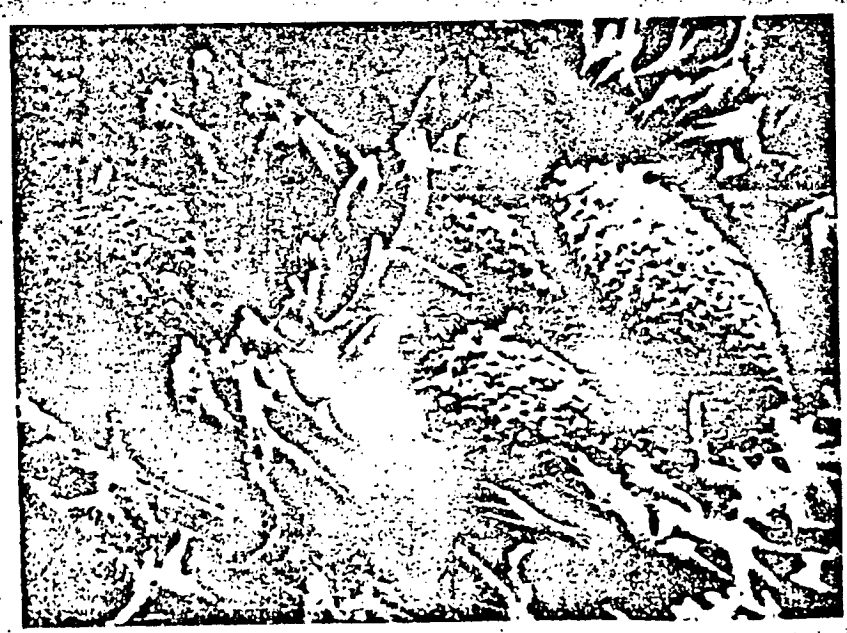

Fig. 6. Epithelium of a bronchiole 225 μm in diameter demonstrating transitional cells and ciliated cells. (Original magnification: × 5,250.)

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B. Brief statement of working hypothesis:

Although pulmonary surfactant is important in expanding the lungs of the newborn, its role in adult lungs, where it is continually produced is not understood. Despite the widespread belief that surfactant, by varying the air-alveolar surface tension, prevents alveoli from collapsing on expiration and from overstretching on inspiration, atelectatic or emphysematous adult human lungs have not been shown to yield abnormal surfactant on pulmonary lavage or by analysis of lung extracts. Our hypothesis is that surfactant provides an important protective function and that maldistribution of surfactant allows cigarette smoke to narrow airways, promote alveolar pleating and damage Type I alveolar cells.

Failure of surfactant to form a ubiquitous layer and convey deposited material from the acinus would expose Type I cell walls to direct and prolonged effects of inhaled substances. The remarkable fact that the vast majority of cigarette smokers do not acquire emphysema suggests that most human lungs are as efficient in protecting their alveoli as tears are in protecting the eyes. To study the chemistry of tears in vitro would reveal very little of their protective function. Thus our insistence that bronchial and alveolar surfaces be studied in situ.

9. Details of experimental design and procedures (append extra pages as necessary)

A. Sources of human lungs, temporal effects and pathology

Human lungs will be obtained from the autopsy service of the Department of Pathology of the University of Colorado Medical Center. A special source will be cadaver transplant donors (kidney and liver) whose usually normal lungs will be made available by the Department of Medicine (Dr. T.L. Petty) and the Division of Biochemistry of the Webb-Waring Lung Institute (Dr. O.K. Reiss).

There are two potential sources of error which must be considered in regard to studies of pulmonary surfactant when the source of the lungs is human beings at autopsy, namely, the time postmortem and the method of sampling surface-active lung fluids. Avery and Mead (1959) reported that in dog lungs there was no significant difference in their surfactant assay results if lungs were studied immediately after death or after refrigeration or freezing for as long as six days. Gruenwald et al (1962) showed that adult human lungs frozen at -20° C and examined six weeks after autopsy were considered satisfactory. Reynolds et al (1965) demonstrated that in lungs of newborn infants obtained within 24 hours of death and stored at -4° to -10° C and then studied within three days, there was no change when surfactant assay was re-examined 12 months after storage. However, the effect of time postmortem on pressure-volume measurements is still not settled. Faridy et al (1966) have shown that dog lobes do not show significant differences in pressure-volume measurements if the lobes were at low temperature for five days, yet Bachofen et al (1970) showed slight changes in pressure-volume curves of cat lungs after three hours after death. Accordingly, we will have to determine the time postmortem effects of the human lungs with which we will be working. We will do this by carrying out repeated pressure-volume and surfactant assay measurements.

Facilities of the Division of Pathology of this Institute will be used to assess the lungs for their normality and to determine the nature of any pathological processes in the tissue. Smoking histories will be documented when available.

B. Surfactant isolation

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For the experiments in Sec. 9G, we will isolate surfactant material by saline lavage (0.9% NaCl) of human postmortem lungs. Serial lavages will be pooled and

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centrifuged at 850 xg to remove alveolar macrophages and other cellular debris. The supernatant will be spun at 10,000 xg to concentrate the surface active material into a pellet. The pellet, which will be resuspended in 0.9% NaCl, is referred to as whole or crude surfactant preparation.

The isolation procedure proposed here is based on experience in this laboratory with rabbit, pig and sheep lung surfactant. Each step will therefore have to be checked for its applicability to the isolation of human material, and this is currently under way in the laboratory of Dr. O.K. Reiss at this Institute. When it becomes desirable to study the purified lipid and protein fractions, such material will be obtained by the zonal density centrifugation methods worked out in this laboratory (Reiss 1970, Gil and Reiss 1973).

The use of micropipettes for direct sampling of surfactant from the alveolar surface and micromethods for the study of its physicochemical properties has been reported by Reifenrath and Zimmermann (1974). Since direct methods have proven decisive in other areas of this field these micromethods seem worth exploring. Staub (1974) has reported the astounding fact that in a related field of tremendous clinical importance (pulmonary edema physiology) "No one except Nitta (1973) has ever obtained direct alveolar fluid." Direct microscmpling methods of surfactant isolation will be used as micromethods of physical and chemical study develop (second and third year).

C. In situ P-V methods

Pressure-volume measurements

Whole human lungs will be ventilated with air and with liquids to determine their quasi-static P-V characteristics; in addition, lobes, segments and sub-segments will be dissected from whole lungs and similarly ventilated. Lungs and portions of lungs will be placed in a volume-displacement plethysmograph similar to the one described by Bachofen et al (1970). This apparatus allows air and liquid pressure-volume curves as well as degassing to be performed without removing the tissue from the plethysmograph.

Our experience with degassing human lungs in the manner often done with animal lungs (Johnson et al 1964), by placing them in a vacuum jar and evacuating the jar until water vaporization and recompression, often resulted in tears and rupture of the parenchyma. This we felt was due to collapse of the small airways trapping gas in the alveoli and eventual rupture of the parenchyma on further evacuation of the vacuum jar. This is overcome by the technique of von Neergaard (1929). A water valve set at 8-10 cm H_2O is inserted between the cannula to the lung and the plethysmograph. With the evacuation pump operating simultaneously both on the bronchus and the space between the lung and plethysmograph, the pressure outside the lung is maintained to about 8-10 cm water lower than in the bronchus. The alveoli thus are in free communication with the bronchial tree until full evacuation, i.e., water vapor pressure, is reached. This will allow filling with air or liquid without any walls being opposed or "stuck together," which filling may well give different results than when collapse is allowed to occur as in conventional degassing. The two techniques will be compared.

An air pressure-volume curve will be done after degassing. The lungs will be inflated to +20 to 22 cm water and deflated to zero pressure three times to standardize volume history. Beginning with the fourth inflation, volume will be

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monitored at 1 cm of water pressure increments until +20 cm of water is reached. Thirty seconds will be allowed between measurements for equilibration of pressure. The same procedure will be followed on the deflation limb of the pressure-volume curve.

As indicated above we will dissect, from normal and pathologic human lungs, portions for direct study of mechanical and other properties connected with surface activity. Isolation of the superior segment of the right lower lobe has already been accomplished (see Fig. 1) along with cannulation of the superior segmental bronchus, B_6, (Bloomer et al 1960). The problem of leaks has been dealt with by gluing the edges of the cut surface to a glass plate with contact cement (Permabond 102, Pearl Chemical Co., Tokyo). Dissection of subsegments of the superior segments of both the right lower and left lower lobes will be carried out with the help of a Magnifying Loupe, Iris forceps, Micro Jameson Scissors and similar surgical equipment (already purchased on the advice of Dr. Melvin Newman). By making use of many representative portions of human lungs we hope to obtain the maximum information possible from a single human lung, especially when the experiments involve ventilation and fixation at various levels of inflation with expensive materials.

D. In situ surfactant distribution

The very recent report of Callas (1974) shows that even after the painstaking work of Gil and Weibel (1969-70) "all published reports indicate failure to demonstrate surfactant as a layer lining the entire alveolar surface." Callas has modified Weibel and Gil's method by attempting to "plaster" the surfactant to the alveolar cells by filling the lungs with agar. In doing this, however, he is compelled to force the agar into a completely collapsed lung (since the air in an inflated lung would prevent the agar from penetrating to alveolar surfaces). Despite the use of his elaborate method, Callas makes the remarkable statement that "no alveoli were found to be completely lined with surfactant."

We believe that no method which introduces a liquid into a lung (especially one that is collapsed) can reveal the normal in situ distribution of surfactant. We therefore propose to use the special degassing technique worked out by von Neergaard (see Sec. 9C) for another purpose. The lungs, instead of being degassed in the usual way in which alveolar surfaces are allowed to fold up, collapse and touch each other (which, we think, must considerably change the distribution of surfactant), are degassed by removal of air molecules without change of lung volume. After this has been accomplished fixation by vaporized or aerosolized glutaraldehyde will be carried out. This method will imitate, to some extent, the formalin steam method of Weibel and Vidone (1961), but will be modified to insure that the lung neither gains nor loses water (Wright et al 1974). In this way the alveolar lining layer will be fixed in situ, its distribution unaffected by processing and fixation artefacts--probably for the first time.

After glutaraldehyde fixation both conventional and electron microscopy methods will be used. Briefly, we will use the ultravioled microscopy technique of Balande and Klause (1964), who concluded that the alveolar lining layer was revealed in guinea pig lungs by fluorescent lines less than one micron thick. The alveolar lining layer of human infants has also been observed using this technique (DeSa 1965). The same tissue fixed in the previously described manner

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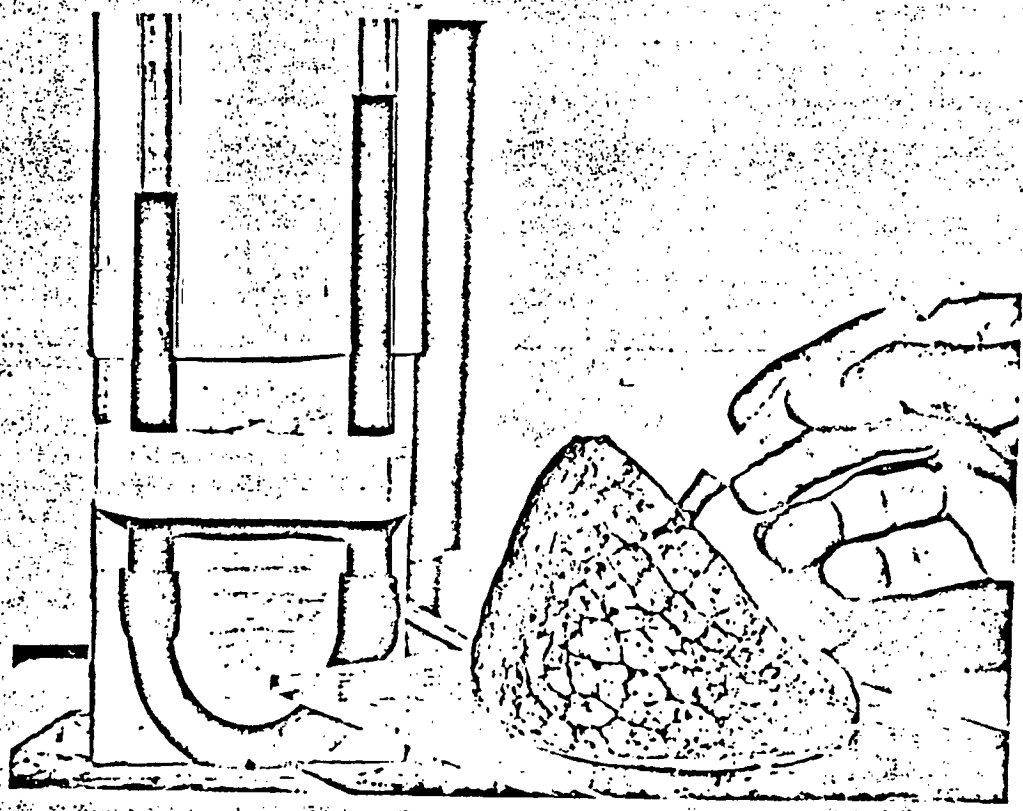

Fig. 1. Superior segment of the right lower lobe of a normal human lung. The cut surface of the posterior basal lobe is resting on a glass plate and glued at the edges. The superior segmental bronchus is cannulated for P-V measurements.

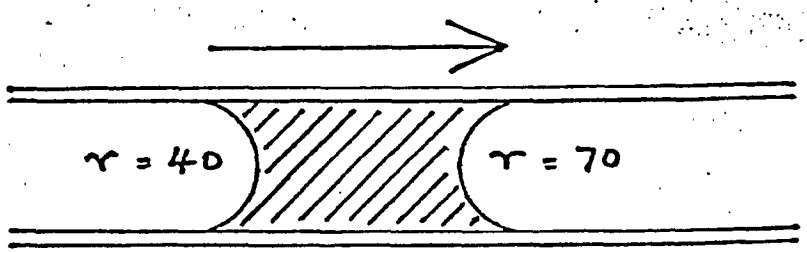

Fig. 2. The fluid is "pulled" in the direction of the arrow by the greater surface energy at the right meniscus. If the right meniscus is facing the trachea or the left the alveoli, surface energies could have a definite role in clearing material from small airways (Schwartz et al., 1964).

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may be used for electron microscopy. Post-fixation methods as described by Callas (1974) will be used in conjunction with the EM facilities of the Division of Cell Biology of this Institute.

E. Methods for the direct study of airway closure mechanisms in the lungs of smokers and non-smokers

Lungs which have had pressure volume curves constructed, as described above, will be dissected to remove a section of the bronchial airways of 2-3 mm in diameter and less. (Surgical advice and assistance will be given to us by Dr. Melvin Newman.) The largest airway will be cannulated while the rest will be tied off with suture. The specimen will be placed in a small plethysmograph equipped with an optical window for microscopic observation similar to that described by Martin and Proctor (1958). The cannulae will be 10 and 20 microliter pipettes, which will be calibrated previously with a Hamilton microliter syringe. A small droplet of water will be placed within the pipette and will serve to mark the volume of air entering the airways. Pressure will be measured by use of a leveling bulb, on a Statham strain-gauge pressure transducer (P23Gb). Thirty second intervals will be allowed for equilibration. A minimum of four complete inflation and deflation pressure-volume curves will be done to check on reproducibility. A similar procedure will be followed when pressure-volume curves of the specimen in various liquids are to be obtained. The plethysmograph will be completely filled with liquid as well as the leveling bulb so that now pressure readings will be obtained by reading the difference between the menisci of the pipette and leveling bulb. The pressures at which the airways open and close will be recorded. From these data we can determine if the airways close at the same or at different pressures in the air filled lung, indicating if surface forces influence small airway closure, and relating these findings to the smoking habits of the patient in life.

Direct microscopy will also be used to determine the relative roles of surface intrinsic and extrinsic forces controlling the behavior of the 0.3-2.0 mm airways of human lobes. Lobes of lungs obtained at surgery or autopsy will have the tracheobronchial tree exposed by dissection with the aid of a dissecting microscope. By cannulating the airways with a blunt needle, pressure-volume curves can be achieved with a microliter syringe and a small volume displacement pressure transducer as described by Macklem et al (1970), the pressures at which different sized airways open and close can be measured and noted with the dissecting microscope. This procedure can be performed by using air, saline, or by flushing with a substance with a known surface tension such as Tween, reflushing with air and repeating the measurements using air. In this manner we can change the surface tension from that normally found in airways to that approaching the tension of the fluid just flushed. Because the geometry and mechanics of dissected airways differs from that of bronchi in the intact lung, we will, in the above methods, be relying on the comparative behavior of airways lined with different materials. If initial volumes and pressures are held constant for each experiment, the differences between P-V relationships should be informative even though the "extrinsic factors" due to lung parenchyma are different than in life (Murtagh et al 1971, Hughes et al 1972, Hughes et al 1973).

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To determine the geometry of closure of airways (lined with naturally occurring surfactant or other materials) as lung tissue volume is reduced, the quantitative methods of Gil (1971) will be used. The bronchi and pulmonary artery supplying the

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lobes or segments of human lungs will be cannulated. By controlling the airway pressure, the lung volume and the flow of fixative, the lungs will be fixed at several points along both inflation and deflation limbs of the P-V curve. Fixation of lungs and appropriate sectioning and microscopic study of the small airways will reveal the morphologic changes they undergo as they "close" during deflation and "open" from a state of closure and should clarify the nature of airway P-V hysteresis (Mead 1973).

To localize portions of airways of particular interest we will, before fixation and/or sectioning, use the facilities of the Magnification Unit in the Department of Radiology. Here a FAXITRON X-ray apparatus (Hewlett-Packard) will enable us to use low energy X-rays. The device has an ultrafine focal spot (0.5 mm) and a beryllium window. Thin pieces of lung will be examined in order to minimize distortion from overlapping of airways. The advantage of this technique is that it allows us to visualize airways as small as 200 μ in diameter and to avoid the use of contrast media after the manner of Murtagh et al (1971).

F. Method for the direct study of airway clearance mechanisms

As Macklem has postulated (1971), based on direct observation of dissected bronchioles, fluid in small airways could form two hemispherical menisci in vivo, one facing the trachea and the other facing the alveoli. His analysis was restricted to considerations of airway stability. However, such fluid menisci are known to have properties which affect fluid movement and which may be important as part of a lung clearance mechanism.

Well-developed formulations (Davies and Rideal 1961, Schwartz et al 1964) relate the movement of a liquid through air-filled horizontal tubes to the surface tension of the liquid and the contact angle between the liquid and tube wall surface. Both theoretically and experimentally, liquid segments of the type visualized by Macklem move through capillary tubes in directions and at rates determined by the difference between the surface tensions at the two menisci. (See Fig. 2). Using the methods discussed under Sec. 9E for dissecting out and studying the bronchi of human lungs, we will investigate, by direct microscopic observation, the effect of naturally present and foreign surface-active materials to the importance of locally varying surface tensions in clearing material from alveolar tissue.

G. Wettability and adhesive properties of pulmonary surfactant on dissected lung tissues and on synthetic materials

As a result of our independent theoretical development, we have concluded that there are several "surface tensions" of importance in the lung. We visualize the surfactant system of the lung as a lining layer (ll) of surfactant "sandwiched" between the air (a) phase and the tissue hypophase (h). The tensions (γ) between the lining layer and air or tissue are given by $\gamma_{ll,a}$ and $\gamma_{h,ll}$ respectively; at places where the tissue is not covered with surfactant the tension is $\gamma_{h,a}$. The relationship among the tensions is given by the equation of Young (1805):

$$\gamma_{h,a} - \gamma_{h,ll} = \gamma_{ll,a} \cos\theta$$

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where θ is the contact angle between the surfactant layer and the tissue. $\theta = 0$ is the condition for complete spreading of surfactant on the tissue.

The purpose of the wetting experiments is to infer the contribution of the tensions at the tissue-air and tissue-surfactant interfaces to the total surface force in the lung, and to determine the spreading characteristics of surfactant on normal and diseased tissue. This is important because, for example, cigarette smoke may alter the tissue while the surfactant appears "normal."

Thin slices of alveolar tissue or small airways, the cut surfaces on which the wettability and adhesion of surfactant will be measured, will be obtained with a tissue slicer or by hand dissection. Chemical fixatives will of course have to be avoided because of possible alteration of the surface properties of the tissue. For the present experiments we will use an automated Wilhelmy film balance of conventional design. Details of construction for the particular balance to be employed have been previously described (Dreher and Wilson 1970). The balance has since been modified to measure γ-A isotherms over a wide range of frequency and to interface with a H-P 7001 x-y recorder. The balance will be suitably mounted to eliminate the effect of vibrations, and cleanliness of chemicals, glassware and surfaces will be in accordance with accepted procedures (Gaines 1966).

Young's equation may be rewritten as $\gamma_{adh} = \gamma_{11,a} \cos\theta$, where $\gamma_{adh} = \gamma_{h,a} - \gamma_{h,11}$ is the adhesion tension. The completeness and reversibility of spreading will be determined by the wetting balance method of Guastalla (1957). If $\theta \neq 0$ then a Wilhelmy plate of platinum measures $\gamma_{11,a} \cos\theta$. Thus, if a plate is constructed of a thin slice of lung tissue (or two slices of tissue with a thin piece of glass or metal sandwiched between them for stability) and the force $\gamma_{11,a}\cos\theta$ transmitted to this tissue plate by a spread film of surfactant on water is measured, then the simultaneous measurement of $\gamma_{11,a}$ with a roughened ($\cos\theta=1$) platinum or glass plate in another part of the film will yield the value of $\cos\theta$ as well as γ_{adh}. In this way the validity of the assumption that surface forces at the air-water interface are similar to the forces on the surface of the lung can be tested in a more direct manner than has heretofore been proposed. For example, if the advancing and receding contact angles are widely different, the γ-Area hysteresis loop traced by the force on the tissue plate would reflect the importance of wettability to the P-V loops in air filled lungs. Similarly, by substituting a liquid for air, the γ-Area loops so obtained should reflect conditions at the interface of a liquid filled lung.

To estimate $\gamma_{h,a}$, the $\cos\theta$ of droplets of low energy liquids (fluorocarbons) of known surface tension ($\gamma_{f,a}$) will be measured on lung tissue which has been rinsed with saline. $\gamma_{h,a}$ may be estimated by application of the equation of Good (1964) and Fowkes (1962):

$$\gamma_{h,a} = \gamma_{f,a} (1 + \cos\theta)^2 / 4\phi^2 \gamma_{f,a}$$

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where ϕ is a numerical constant. Lung tissue strips will be mounted horizontally in a small plexiglas chamber with ports for evacuation or saturation with gas. The θ's will be measured with a cathetometer telescope and a gionometer eyepiece. The magnitude of $\gamma_{h,a}$ and $\gamma_{h,11}$ (which is now known from $\gamma_{h,a}$ and the previously measured γ_{adh}) relative to $\gamma_{11,a}$ will reflect the importance of the tissue stress in the total retractive force in the lung.

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Methods of measuring the adhesive force between similar or dissimilar surfaces coated with a liquid film have been known for many years (Budgett 1912). These forces are often quite high, thus it is worthwhile to determine if reductions in the adhesive force can be effected by pulmonary surfactant. This problem will be approached by using a duNouy tensiometer to measure the vertical force required to separate plates of synthetic materials (glass, metal, teflon) or slices of lung tissue coated with surfactant. Since lung surfactant is known to be viscoelastic (Kott et al 1974), the results will depend on the rate of separation of the surfaces. Initially the experiments will be confined to quasi-static conditions. By comparing the work required to separate lung tissue in air with that required in saline, the idea is to show that the known reduction in retractive force in liquid filled lungs relative to air filled lungs can be explained by an alternate mechanism of surfactant operation. For example differences in the air and saline work of adhesion would indicate that surfactant could produce the difference seen between air and liquid P-V curves without significant changes in lung area by stretching of the alveolar surface, but rather by a simple unfolding of alveolar pleats.

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10035461 34

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Weibel ER, Gil J: Electron microscopic demonstration of an extracellular duplex lining layer of alveoli. Respir Physiol 4: 42, 1968.

Weibel ER: Morphological basis of alveolar capillary gas exchange. Physiol Rev 53: 419, 1973.

Weibel ER, Untersee P, Gil J, Zulauf M: Morphometric estimation of pulmonary diffusion capacity. VI. Effect of varying positive pressure inflation of air spaces. Respir Physiol 18: 285, 1973.

Williams R: Computer analysis and model of the properties of pulmonary surfactant: effects of nitrogen dioxide. Ph.D. Dissertation, Pennsylvania State Univ, University Microfilms, Ann Arbor, Mich, 1970.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

A 600-square foot laboratory in the basement of the Webb-Waring Lung Institute (W-WLI) is available for the postmortem lung mechanics studies and for the isolated surfactant studies. A pathology laboratory equipped for inflation-fixation and cutting of lungs, preparation of histologic sections and long-term storage of fixed lung slices and histologic material is available on the fourth floor of W-WLI.

Major items of equipment availabor for this work at W-WLI include:
Electronics for Medicine IR-r recording equipment
Hewlett-Packard 7001 x-y recorder
Beckman GC2-A gas chromatograph
Electronics for Medicine DR-8 and assorted transducers
Monoghan respirator
Harvard respiration pump
Beckman Spinco Model L Ultracentrifuge
International Refrigerated Centrifuge Model PR-2
Sorvall RC2-B refrigerated centrifuge
Mettler M-5 microbalance
Automatic quartz bi-distillation unit for production of pure water
Automated Wilhelmy surface balance modified for variable frequency and temperature controlled gas studies
Collins spirometer
Phillips 300 Electron Microscope

The Department of Radiology is equipped with Faxitron X-ray facilities and auxiliary equipment.

11. Additional facilities required:

None

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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3a

CURRICULUM VITAE

GILES F. FILLEY, M.D.

June 1972

Birth Date: REDACTED

Marital Status: REDACTED

Children:

Education: Kent School, Kent Conn. Graduated R -- High School
Williams College, Williamstown, Mass. Graduated REDACTED
Johns Hopkins University, Baltimore, Md. Graduated REDACTED

Internship: Johns Hopkins Hospital, Baltimore, Md. 1942-1943.

Residencies: Lawrason Brown Fellow, Department of Physiology, The Edward L. Trudeau Foundation, Saranac Lake, New York 1943-1944.

Assistant in Medicine, Johns Hopkins University. 1944-1945.

Instructor in Medicine and John D. Archbold Fellow in Medicine, Johns Hopkins University. 1945-1946.

Assistant Resident Physician, Medical Ward Service, Barnes Hospital, St. Louis, Mo. 1946-1947.

Positions: Associate Physiologist, Department of Physiology, The Edward L. Trudeau Foundation, Saranac Lake, New York. 1947-1953.

Director, Department of Physiology, The Trudeau-Saranac Institute, Saranac Lake, New York. 1953-1955.

Assistant Professor of Medicine, University of Colorado School of Medicine, Denver, Colorado. 1955-1959.

Associate Professor of Medicine, University of Colorado School of Medicine, Denver, Colorado. 1959-1969.

Professor of Medicine, University of Colorado School of Medicine, Denver, Colorado. 1969-Present.

Clinical Physiologist, Chief-Division of Physiology, Webb-Waring Lung Institute, Denver, Colorado. 1955-Present.

Memberships in Societies:

REDACTED REDACTED

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Consulting Positions: Denver General Hospital
Fitzsimons Army Hospital
Veterans Administration Hospital

10035461 38

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RESUME

George W. Paul

REDACTED

Telephone:

(303) 394-8731 (Office)

PERSONAL

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RESEARCH INTERESTS

Biomedical applications of surface chemistry, structure of water and liquid solutions, membrane phenomena, thermodynamics (equilibrium and non-equilibrium), transport processes. Have reviewed about 400 papers in these areas. Long range: Teaching or research institute.

EDUCATION

University of Missouri (Columbia): B.S. R M.S. R Ph.D. R all chemical engineering. G.P.A.: R

M.S. Thesis: "Interfacial Free Energy in Binary and Ternary Systems."

Ph.D. Comprehensive Problem: "An Investigation of Solute Diffusion in Polymer Solutions."

Ph.D. Dissertation: "Interfacial Tension in the Critical Region."

EXPERIENCE

Summer 1963: U.S. Gypsum Co., Mexico, Missouri. Polymer coating technology.

Summer 1964 and 1965: Nalco Chemical Co., Chicago, Illinois. Correlation of kinetics of co-polymerization reactions.

Summer 1966 and 1967: Research Assistant, Engineering Experiment Station, University of Missouri, Columbia, Missouri. Undergraduate instruction in techniques of digital computation.

10035461 39

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8/69 to 11/72: Standard Oil Co. (Ind.) Research Center, Tulsa, Oklahoma. Surfactants and biopolymers (polysaccharides). Evaluation of experiments on micellization, non-equilibrium adsorption, flow through porous media.

11/72 to present: Postdoctoral Research Fellow, Webb-Waring Lung Institute, University of Colorado Medical Center, Denver, Colorado. My present work is focused on understanding the physical chemistry of the lining of human lungs. The surface of mammalian lungs in general is composed of a special fluid of peculiar surface properties. It is known that some diseases result directly from disturbances in these surface properties. It is likely that even more common diseases such as emphysema involve changes in the chemistry of these substances and my work is devoted to determining these changes.

ACTIVITIES

1968, Teen Club Worker, Columbia, Missouri. Avid tennis player, reader of contemporary history.

PROFESSIONAL ORGANIZATIONS

REDACTED REDACTED REDACTED

HONORS AND AWARDS

Green Scholarship, Curators Scholarship, American Chemical Society Research Fellowship, NASA Fellowship, Omicron Delta Kappa, Pi Omicron Sigma.

REFERENCES

Professor Marc deChazal, Department of Chemical Engineering, University of Missouri, Columbia, Missouri 65201; Dr. J.T. Ryan, Department of Chemical Engineering, University of Alberta, Edmonton, Alberta, Canada; Dr. P.D. Shoemaker, Monsanto Company, 800 N. Lindbergh, St. Louis, Missouri.

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Resume

G. Wayne Silvers

Born: REDACTED

Education: University of Colorado, Boulder, Colorado
B.A., Zoology, 1963.

Appointments: Research Associate, University of Colorado Medical Center, Denver, Colorado, 1972 - present.

Research Physiologist, University of Colorado Medical Center, 1967-1972

Research Technician, University of Colorado Medical Center, 1963-1967.

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CURRICULUM VITAE

Melvin M. Newman, M.D.

Place and Date of Birth: REDACTED

College: University of Chicago, R

Medical School: University of Chicago, R B. S. R, M. D. R

Honor Societies: Phi Beta Kappa, Alpha Omega Alpha, Sigma Xi

Rotating Internship: University of Chicago Clinics, September 1944 - June 1945

Residency Training: University of Chicago Clinics, October 1946 - July 1952
General and Thoracic Surgery

Teaching and Research Appointments: Instructor in Surgery, University of Chicago, July 1950-- June 1952

National Research Council Fellow, University of Chicago
July - October 1952 (Recalled to Active Duty, U.S. Navy)

Assistant Professor of Surgery, State University of New York
College of Medicine at Brooklyn, July 1954 - March 1956

Associate Professor, March 1956 - August 1959

Director of Surgery, National Jewish Hospital at Denver,
September 1959 to August 1968

Clinical Associate Professor of Surgery, University of Colorado,
1961 to 1968

Associate Professor of Surgery (Full-time), University of Colorado,
1968 to present

Certification: American Board of Surgery - 1951; Board of Thoracic Surgery - 1953

Military Service: Lt. J.G. MC USNR, July 1945 - August 1946
Lt. MC USNR, October 1952 - July 1954 (Chief of Thoracic Surgery,
U. S. Naval Hospital, St. Albans, N.Y., August 1953 - July 195

Marital Status: Married September 11, 1949, Joyce Kligerman Newman, Two children

Lincensure: Illinois - 1946; New York - 1955; Colorado - 1960

Professional Societies: American College of Surgeons, American College of Chest Physicians, American Association for Thoracic Surgery, American Thoracic Association, American Society for Artificial Internal Organs, Society of University Surgeons, Societe Internationale de Chirurgie, Society of Thoracic Surgeons.

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13. Publications

Giles F. Filley, M.D.

Wagner, W.W., Barker, D.B., and Filley, G.F.: A Photographic Method for Quantitating Blood Flow in the Pulmonary Microcirculation. J. Biological Photographic Assoc. 35: 95, 1967.

Filley, G.F.: Acid-Base and Blood Gas Regulation. Lea & Febiger, Philadelphia, March 1971.

Horsfield, D., Dart, G., Olson, D.E., Filley, G.F., and Cumming, G.: Models of the Human Bronchial Tree. J. Appl. Physiol. 31: 207, 1971.

Silvers, G.W., Maisel, J.C., Petty, T.L., Filley, G.F., and Mitchell, R.S.: Central Airway Resistance in Excised Emphysematous Lungs. Chest 61: 603, 1972.

Olson, D.E., Sudlow, M.F., Horsfield, K., and Filley, G.F.: Convective Patterns of Flow During Inspiration. Arch. Int. Med. 131: 51, 1973.

George W. Paul, Ph.D.

Paul, G.W., and Marc de Chazal, L.E.: Interfacial Tensions in Ternary Liquid-Liquid Systems. J. Chem. Engr. Data 12: 105, 1967.

Paul, G.W., and Marc de Chazal, L.E.: Correlation of Interfacial Free Energy in Binary and Ternary Systems. Ind. Engr. Chem. Fundamentals 8: 104, 1969.

Shoemaker, P.D., Paul, G.W., and Marc de Chazal, L.E.: Surface Tension of Simple Liquids from the Radial Distribution Function. J. Chem. Phys. 52: 491, 1970.

Paul, G.W., and Froning, H.R.: Salinity Effects in Micellar Flooding. J. Pet. Tech. 24: 957, 1973.

G. Wayne Silvers

Silvers, G.W., Maisel, J.C., Petty, T.L., Filley, G.F., and Mitchell, R.S.: Central Airway Resistance in Excised Emphysema Lungs. Chest 61: 603, 1972.

Maisel, J.C., Silvers, G.W., George, M.S., Dart, G.A., Petty, T.L., and Mitchell, R.S.: The Significance of Bronchial Atrophy. Am. J. Path. 67: 371, 1972.

Silvers, G.W., Maisel, J.C., Petty, T.L., and Mitchell, R.S.: Reduction in Peripheral Airway Resistance in Excised Emphysematous Lungs. Proc. 15th Annual Aspen Emphysema Conf. Chest 63: 32S, 1973.

Silvers, G.W., Maisel, J.C., Petty, T.L., Filley, G.F., and Mitchell, R.S.: Increase of Flow in Excised Emphysematous Lungs Following Lavage with Acetylcysteine or Salina. Am. Rev. Resp. Dis. 110: 170, 1974.

Silvers, G.W., Maisel, J.C., Petty, T.L., Filley, G.F., and Mitchell, R.S.: Flow Limitation During Forced Expiration in Excised Human Lungs. J. Appl. Physiol. 36: 737, 1974.

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14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Giles F. Filley, M.D. (Princip.l Investigator)	30%	—
George W. Paul, Ph.D. Postdoctoral Res. Fellow	100%	
G. Wayne Silvers, Research Associate	50%	
Melvin M. Newman, M.D., Consultant	Not stipulated	--
Technical		
Marlyce George, Research Technologist	50%	- -
Fringe Benefits		
Sub-Total for A		

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B. Consumable supplies (by major categories)

Chemicals & fixatives	750
Valves, tubing, glassware	350
X-ray room use fee & supplies	450
Recorder paper & developer	300
Suction filter	25
Sub-Total for B	1,875

C. Other expenses (itemize)

Publication costs	500
Maintenance and Modification of Equipment	1,000
Travel	500
Sub-Total for C	2,000
Running Total of A + B + C	35,573

D. Permanent equipment (itemize)

1 ea Model 1210 Harvard Peristaltic Pump	625
1 ea Model 550 Harvard Pump speed modulator	225
1 ea Large speciman plethysmograph	200
1 ea duNouy Tensiometer	495
1 ea Cathetometer telescope and stand	370
1 ea Gionometer Eyepiece	237
1 ea Statham transducer	350
1 ea Universal illuminator	111
Sub-Total for D	2,613
E	5,336
Total request	43,522

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	1,500	2,000	--	5,664	43,427
Year 3		1,650	1,000	--	5,955	45,656

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Cardiopulmonary Physiology	USPHS 3 T01 HL05450-14S1	36,423	4/1/74 - 6/30/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Human Pulmonary Surfactant Function in Situ	USPHS 1 R01 HL 17544-01A1	42,245	4/1/75 - 3/31/76
Surface Chemistry and Physics of Pulmonary Surfactant (Young Investigator) G.W. Paul	USPHS 1 R23 HL 18353-01	15,000	7/1/75 - 6/30/76

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Giles F. Filley, M.D.

Signature [signature] Date Jan 21 '75

Telephone REDACTED
Area Code Number Extension

Checks payable to

iversity of Colorado Medical Center
Office of Grants & Contracts

Mailing address for checks

4200 East Ninth Avenue

Denver, Colorado 80220

Responsible officer of institution

Typed Name Harry P. Ward, M.D.

Title Acting Vice President for Health Affairs

Signature [signature] Date 1-23-75

Telephone REDACTED
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